Net flux of amino acids across the portal-drained viscera and liver of the ewe during abomasal infusion of protein and glucose[1,2]

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ABSTRACT: The objective of the study was to measure net AA flux rates across the portal-drained viscera (PDV) and liver in the presence and absence of abomasal glucose infusion. Decreasing the fraction of AA metabolized by the mucosal cells may increase the fraction of AA being released into the blood. A potential mechanism to reduce AA catabolism by mucosal cells is to provide an alternative source of energy. We hypothesized that increasing glucose flow to the small intestine would increase net appearance of AA across the PDV. Eighteen mature ewes with sampling catheters were placed on study. The experimental design was a split-plot with a complete randomized design on the whole-plot and a Latin-square subplot with 5 periods and incremental levels of protein infusion. One-half of the ewes received abomasal glucose infusions (3.84 g/h), and all ewes received each of 5 protein abomasal infusion levels over 5 periods (0, 2.6, 5.2, 7.8, and 10.5 g/h). Net PDV release of isoleucine, leucine, methionine, phenylalanine, aspartate, glutamate, glutamine, proline, serine, and tyrosine increased linearly with increased protein infusion, and net PDV release of histidine, lysine, threonine, valine, alanine, and glycine did not differ with protein infusion. Net hepatic glucose release decreased with glucose infusion. With the exception of histidine, phenylalanine, and valine, net hepatic AA uptake increased linearly with increased delivery of AA to the liver. Glucose infusion increased the hepatic lysine and valine uptake and decreased phenylalanine uptake. Based on the observations in the current study, we reject our hypothesis that glucose can spare AA metabolism by PDV tissue. Our findings suggest that hepatic gluconeogenesis can be increased in the presence of increased AA delivery to the liver and that hepatic gluconeogenesis can be decreased with increased absorption of dietary glucose. Our findings support the concept that for most AA, hepatic transport of AA can be described by mass action kinetics; however, the rates of hepatic uptake of specific AA are upregulated directly or indirectly by elevated glucose.

Key words: amino acid, intestine, liver, sheep

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INTRODUCTION

Providing adequate protein is important to allow ruminants to produce milk, meat, and fiber at a level to support efficient animal production. However, providing excessive nitrogen to animals in confinement potentially contributes to nitrogen contamination of air and water from animal waste (Cole et al., 2005). Improved efficiency of N utilization by the animal may improve production efficiency and reduces the negative impact of animal excretions on the environment. Sheep and cattle absorb feed N as ammonia, peptides, and AA (Gilbert et al., 2008). Amino acids are used for synthesis of proteins, as precursors for gluconeogenesis, and as a source of energy (Freetly et al., 1993; Hanigan et al., 2004b). Small intestine mucosal cells absorb dietary AA from the lumen of the small intestine. Amino acids that enter the intestinal epithelium are used for protein synthesis (structural and secretory), catabolized for energy, or released into the blood through the basal membrane. Studies in sheep (MacRae et al., 1997) and in pigs (Stoll et al., 1998) reported that approximately one-third of the AA absorbed by mucosal cells are metabolized within the cells and are never released into the blood. Of those AA that are metabolized, 60% are most likely catabolized (Stoll et al., 1998), which suggests that 20% of the absorbed AA are catabolized for energy within the mucosal cells. Nitrogen from catabo-

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Table 1. Blood flow, oxygen consumption, and net release of glucose and urea-N by splanchnic tissue during abomasal protein infusion with and without glucose[1]

Item	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Blood flow, L/h																	
Arterial	—	—	22.3	3.7	16.5	3.7	9.21	3.7	13.5	3.7	12.7	3.7	0.16	<0.001	0.37	0.003	<0.001[5]
Portal	—	—	122.4	4.8	119.9	4.8	122.5	4.8	116.8	4.8	114.9	4.8	0.97	0.19	0.12	0.48	0.04
Hepatic													0.28	0.004	0.006	0.40	I = 0.005
Control	—	—	131.0	6.9	130.1	6.9	135.4	6.9	124.7	6.9	128.9	6.9					
Glucose	—	—	155.5	6.5	142.4	6.5	132.5	6.5	136.4	6.5	128.1	6.5					
Oxygen, mmol/h																	
PDV[6]	148.3	2.9	—	—	—	—	—	—	—	—	—	—	0.89	0.82	0.75	0.23	0.93
Hepatic	—	—	137.8	8.7	121.9	8.7	121.5	8.7	125.1	8.7	135.6	8.7	0.40	0.37	0.92	0.047	0.96
Splanchnic	276.6	4.8	—	—	—	—	—	—	—	—	—	—	0.50	0.94	0.56	0.53	0.94
Whole blood, m*M*																	
Glucose																	
Arterial													<0.001	0.001	0.002	0.25	I <0.001
Control	—	—	2.61	0.07	2.73	0.07	2.86	0.07	2.94	0.07	2.99	0.07					
Glucose	—	—	3.06	0.07	3.22	0.07	3.11	0.07	3.11	0.07	3.13	0.07					
Portal													<0.001	0.003	<0.001	0.03	I <0.001
Control	—	—	2.49	0.07	2.64	0.07	2.76	0.07	2.84	0.07	2.87	0.07					
Glucose	—	—	3.10	0.07	3.26	0.07	3.14	0.07	3.15	0.07	3.07	0.07					
Hepatic													<0.001	<0.001	0.02	0.47	I = 0.002
Control	—	—	2.95	0.05	3.09	0.05	3.11	0.05	3.15	0.05	3.23	0.05					
Glucose	—	—	2.70	0.08	2.84	0.08	2.97	0.08	3.05	0.08	3.11	0.08					
Urea-N																	
Arterial	—	—	6.9	0.4	7.4	0.4	7.6	0.4	7.6	0.4	8.0	0.4	0.14	0.08	0.60	0.69	0.008
Portal	—	—	6.8	0.4	7.3	0.4	7.5	0.4	7.5	0.4	7.9	0.4	0.14	0.08	0.62	0.72	0.007
Hepatic	—	—	7.2	0.4	7.9	0.4	8.2	0.4	8.1	0.4	8.5	0.4	0.09	0.05	0.71	0.50	0.005
Control	8.5	0.4	—	—	—	—	—	—	—	—	—	—					
Glucose	7.4	0.4	—	—	—	—	—	—	—	—	—	—					
Release, mmol/h																	
Glucose																	
PDV	—	—	−5.5	1.5	−3.4	1.5	−4.8	1.5	−2.9	1.5	−8.7	1.5	<0.001	0.05	0.53	0.03	0.21
Control	−12.3	0.9	—	—	—	—	—	—	—	—	—	—					
Glucose	2.2	0.9	—	—	—	—	—	—	—	—	—	—					
Hepatic	—	—	19.7	2.0	17.4	2.0	20.1	2.0	19.5	2.0	29.9	2.0	0.007	<0.001	0.39	0.004[5]	<0.001
Control	24.1	1.3	—	—	—	—	—	—	—	—	—	—					
Glucose	18.6	1.2	—	—	—	—	—	—	—	—	—	—					
Splanchnic													<0.001	0.002	0.18	I = 0.03	<0.001
Control	—	—	6.6	2.0	10.6	2.0	12.0	2.0	11.4	2.0	15.6	2.0					
Glucose	—	—	21.0	1.9	17.1	1.9	18.3	1.9	20.9	1.9	26.0	1.9					
Urea-N																	
PDV													0.29	0.55	0.02	0.18	I = 0.008
Control	—	—	−16.6	2.5	−21.7	2.5	−16.1	2.5	−15.3	2.5	−11.1	2.5					
Glucose	—	—	−12.4	2.5	−10.8	2.5	−14.4	2.5	−16.9	2.5	−14.5	2.5					

Continued

Table 1 (Continued). Blood flow, oxygen consumption, and net release of glucose and urea-N by splanchnic tissue during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h								*P*-value for ANOVA[3]			Contrast *P*-value[4]	
Item	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Hepatic	—	—	51.0	3.8	57.4	3.8	66.2	3.8	64.9	3.8	70.2	3.8	0.21	0.002	0.41	I = 0.07	<0.001
Splanchnic	—	—	35.6	3.6	41.2	3.6	50.2	3.6	48.4	3.6	57.6	3.6	0.48	<0.001	0.96	0.78	<0.001

[1]Treatments consisted of abomasal glucose infusion of 3.84 g/h (glucose) or no glucose infusion (control) and 5 levels of abomasal protein infusion that consisted of soy protein and cysteine in a ratio of 0.09 g of cysteine per gram of soy protein.

[2]Overall algebraic mean and SE for all observations n = 90 for PDV release and arterial and portal vein concentration, and 85 for hepatic and splanchnic releases and hepatic vein concentrations. Treatment means are least squares means and SE.

[3]ANOVA was analyzed as a mixed model with glucose treatment (glucose), protein infusion level (protein), the interaction of glucose and protein (G × P), and period as fixed effects and animal with treatment as a random effect. Nine animals each were in the control and glucose treatment. Eight observations were observed for hepatic concentration, hepatic release, and splanchnic release in the control treatment.

[4]Probability estimates for the quadratic and linear orthogonal contrast for protein infusion. The values labeled as equal to I are the *P*-values associated with interaction between protein and glucose treatment when the interaction was significant.

[5]Main effect differed ($P < 0.05$), and the interaction between protein and glucose treatment tended to differ ($0.05 < P < 0.10$).

[6]PDV = portal-drained viscera.

lized AA is transported to the liver where urea is synthesized. Synthesis of urea and its subsequent excretion requires additional energy. Decreasing the fraction of AA metabolized by the mucosal cells may increase the fraction of AA being released into the blood. One potential mechanism to reduce AA catabolism by mucosal cells is to provide an alternative source of energy. Enteral glucose oxidation increased in the neonatal pig when dietary protein was decreased (van der Schoor et al., 2001), suggesting that conversely increasing glucose supply may decrease AA oxidation. We hypothesized that increasing glucose flow to the small intestine would increase net appearance of AA across the portal-drained viscera (**PDV**). The objective of the study was to measure net AA flux rates across the PDV and liver in the presence and absence of abomasal glucose infusion.

MATERIALS AND METHODS

The experiment was conducted to conform with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and was approved by the US Meat Animal Research Center Animal Care and Use Committee.

Eighteen multiparous (5.4 ± 0.3 yr) polled Dorset ewes (71.7 ± 0.7 kg) were individually penned (1.17 m^2) in a temperature-controlled room (19°C). Ewes were fed a pelleted diet (95% brome hay and 5% soybean meal, as DM) at 52.2 g/BW $kg^{0.75}$. The diet contained 10% CP and had a calculated ME of 1.96 Mcal/kg (DM basis). The diet provided adequate ME and protein to exceed maintenance requirements (NRC, 2007). Ewes were fed a single meal daily at 1500 h. Ewes had ad libitum access to water and a mineral supplement (65.012% NaCl, 16.380% CaO_3, 16.199% $CaHPO_4$, 0.106% $FeSO_4$, 0.058% ZnO, 0.043% Mn_3O_4, 0.001% $C_2H_8N_2$2HI, 0.001% $CoCO_3$, and 2.200% soybean oil).

Catheters were surgically placed in the hepatic portal vein, a branch of the hepatic vein, a mesenteric vein, and the abdominal aorta as described by Ferrell et al. (1992). In addition to the catheters placed for sampling blood, a catheter was placed in the abomasum to facilitate infusion of glucose and protein. The abomasal catheter consisted of a 1-m length of Tygon tubing (1.59 mm i.d., 3.18 mm o.d.; Saint-Gobain Performance Plastics, Akron, OH) that had a pair of cuffs constructed of Tygon that formed a 5-cm tip. A small hole was created by dissection in the fundus of the abomasum, and the tip of the catheter was inserted into the abomasum. The catheter was sutured to the abomasum using a purse string suture at the cuff and an additional suture on the surface of the abomasum.

Ewes were sorted on age and BW and randomly assigned to 1 of 2 treatments within age and BW. Treatments were glucose infusion into the abomasum (3.84 g/h) or no glucose infusion into the abomasum (control). Glucose infusion rate equaled the expected net hepatic release rate (Freetly and Klindt, 1996). Sample

Table 2. Net release of essential AA (mmol/h) by splanchnic tissue during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
AA	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Histidine[5]																	
PDV[6]	0.80	0.29	—	—	—	—	—	—	—	—	—	—	0.09	0.25	0.39	0.37	0.71
Hepatic													0.69	0.28	0.35	I = 0.04	0.77
Control	—	—	−0.32	1.26	0.28	1.18	−0.44	1.26	−0.67	1.20	−0.53	1.26					
Glucose	—	—	1.30	1.11	0.31	1.11	−2.40	1.11	−1.40	1.26	2.04	1.18					
Splanchnic													0.05	0.47	0.38	0.17	0.91
Control	−0.05	0.49	—	—	—	—	—	—	—	—	—	—					
Glucose	1.35	0.46	—	—	—	—	—	—	—	—	—	—					
Isoleucine																	
PDV	—	—	0.57	0.15	0.78	0.15	1.04	0.15	1.01	0.15	1.29	0.15	0.51	0.01	0.18	0.76	<0.001
Hepatic	−0.27	0.07	—	—	—	—	—	—	—	—	—	—	0.52	0.16	0.83	0.24	0.15
Splanchnic	0.64	0.09	—	—	—	—	—	—	—	—	—	—	0.88	0.34	0.61	0.71	0.11
Leucine																	
PDV	—	—	0.79	0.24	1.14	0.24	1.47	0.24	1.50	0.24	1.93	0.24	0.49	0.02	0.29	0.88	<0.001
Hepatic	−0.45	0.11	—	—	—	—	—	—	—	—	—	—	0.43	0.18	0.67	0.24	0.16
Splanchnic	0.88	0.14	—	—	—	—	—	—	—	—	—	—	0.87	0.30	0.71	0.64	0.10
Lysine[7]																	
PDV	1.74	0.34	—	—	—	—	—	—	—	—	—	—	0.08	0.24	0.73	0.65	0.15
Hepatic	—	—	1.12	0.66	0.42	0.64	−1.95	0.66	−1.01	0.66	−1.19	0.68	0.10	0.01	0.72	0.09	0.006
Splanchnic	1.43	0.68	—	—	—	—	—	—	—	—	—	—	0.38	0.28	0.31	0.23	0.07
Methionine																	
PDV	—	—	0.24	0.04	0.27	0.04	0.32	0.04	0.30	0.04	0.39	0.04	0.14	0.15	0.65	0.74	0.02
Hepatic	—	—	−0.10	0.03	−0.12	0.03	−0.21	0.03	−0.20	0.03	−0.21	0.03	0.16	0.03	0.44	0.27	0.004
Splanchnic	0.13	0.02	—	—	—	—	—	—	—	—	—	—	0.75	0.86	0.73	0.49	0.71
Phenylalanine																	
PDV	—	—	0.64	0.15	0.96	0.15	1.18	0.15	1.16	0.15	1.52	0.15	0.23	0.003	0.31	0.78	0.001
Hepatic	—	—	−0.72	0.13	−0.74	0.13	−1.25	0.13	−1.24	0.13	1.20	0.13	0.14	0.003	0.62	0.19	0.001
Splanchnic	0.05	0.07	—	—	—	—	—	—	—	—	—	—	0.99	0.30	0.69	0.64	0.27
Threonine																	
PDV	0.82	0.11	—	—	—	—	—	—	—	—	—	—	0.43	0.49	0.07	0.54	0.09
Hepatic													0.04	0.19	0.65	0.82	0.07
Control	−0.89	0.17	—	—	—	—	—	—	—	—	—	—					
Glucose	−0.36	0.16	—	—	—	—	—	—	—	—	—	—					
Splanchnic	0.17	0.14	—	—	—	—	—	—	—	—	—	—	0.24	0.35	0.20	0.66	0.90
Valine																	
PDV	1.03	0.14	—	—	—	—	—	—	—	—	—	—	0.55	0.54	0.12	0.63	0.13

Continued

Table 2 (Continued). Net release of essential AA (mmol/h) by splanchnic tissue during abomasal protein infusion with and without glucose[1]

AA	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h								*P*-value for ANOVA[3]			Contrast *P*-value[4]	
	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Hepatic	−0.55	0.18	—	—	—	—	—	—	—	—	—	—	0.24	0.22	0.84	0.24	0.41
Splanchnic	0.42	0.20	—	—	—	—	—	—	—	—	—	—	0.43	0.32	0.60	0.61	0.63

[1]Treatments consisted of abomasal glucose infusion of 3.84 g/h (glucose) or no glucose infusion (control) and 5 levels of abomasal protein infusion that consisted of soy protein and cysteine in a ratio of 0.09 g of cysteine per gram of soy protein.

[2]Overall algebraic mean and SE for all observations n = 90 for PDV and 85 for hepatic and splanchnic unless otherwise noted. Treatment means are least squares means and SE.

[3]ANOVA was analyzed as a mixed model with glucose treatment (glucose), protein infusion level (protein), the interaction of glucose and protein (G × P), and period as fixed effects and animal with treatment as a random effect. Nine animals each were in the control and glucose treatment. Eight observations were observed for hepatic and splanchnic release in the control treatment.

[4]Probability estimates for the quadratic and linear orthogonal contrast for protein infusion. The values labeled as equal to I are the *P*-values associated with interaction between protein and glucose treatment when the interaction was significant.

[5]Missing observations for 1 control animal at protein infusion levels 0, 5.2, and 10.5 g/h and 2 glucose animals at 7.8 g/h and 1 glucose animal at 10.5 g/h.

[6]PDV = portal-drained viscera.

[7]Missing observation for 1 control animal at protein infusion levels 0, 5.2, and 10.5 g/h and 1 glucose animal at 7.8 and 10.5 g/h.

collection began 5 wk after surgery and consisted of 5 collection periods. During each collection period, ewes received 1 of 5 protein infusions into the abomasum, such that over the course of the experiment each ewe received each of the 5 levels of protein infusion. The protein infusions consisted of a combination of an isolated soy protein (Ardex F Dispersible, Archer Daniels Midland Company, Decatur, IL) and cysteine. The 5 infusion levels delivered the following amounts of protein each hour: (0 g of soy protein + 0 g of cysteine), (2.4 g of soy protein + 0.216 g of cysteine), (4.8 g of soy protein + 0.432 g of cysteine), (7.2 g of soy protein + 0.648 g of cysteine), and (9.6 g of soy protein + 0.864 g of cysteine). Abomasal infusions were delivered at a rate of 1.5 mL/min using a peristaltic pump. Glucose was mixed with the infusate for the ewes receiving the glucose treatment. Protein levels ranged from approximately one-half to 2 times the protein provided by the basal diet. Ewes were allowed 2 wk between collection periods.

On the day that net flux measurements were taken, abomasal infusions of protein with glucose (glucose treatment) and abomasal infusions of protein without glucose (control) were started at 0800 h (17 h after the meal). Abomasal infusions continued throughout the rest of the day. Three hours after abomasal infusions were started, a 15-mL bolus of para-amino hippuric acid (**PAH**; 0.15 *M*) was given via the mesenteric vein. The bolus was followed by a constant infusion of PAH (0.8 mL/min). Four hours after abomasal infusions were initiated, blood samples were collected into heparinized syringes (9 mL) from the aortic, hepatic portal venous, and hepatic venous catheters. An additional sample (1 mL) was anerobically collected from each catheter into a heparinized syringe to determine hemoglobin concentration and oxygen saturation of hemoglobin (Hemoximeter Model OSM 3, Radiometer America, Westlake, OH). Sets of blood samples were collected every 30 min for a total of 5 sets of samples.

Fresh blood samples were analyzed for ammonia N, urea-N, PAH, and lactate as described by Freetly and Ferrell (1998). Blood glucose (glucose oxidase, EC 1.1.3.4), glutamate (glutamate oxidase, EC 1.4.3.11), and glutamine (glutaminase, EC 3.5.1.2 and glutamate oxidase, EC 1.4.3.11) concentrations were determined on a membrane-immobilized system (model 2700, Yellow Spring Instrument Co., Yellow Springs, OH). Blood AA were analyzed according to the procedure of Calder et al. (1999). Blood flow was calculated using PAH in the indicator-dilution technique (Katz and Bergman, 1969b). Net fluxes of nutrients were calculated by multiplying the concentration difference between vessels by the blood flow rate (Katz and Bergman, 1969a). Blood chemistries were analyzed for each replicate sample within ewe and period, and individual fluxes were calculated for each replicate sample. Replicate samples were averaged within period and ewe. Replicates that exceeded 1 SD of the within-ewe period mean and negative blood flows were removed from the data set.

Table 3. Net release of nonessential AA (mmol/h) by splanchnic tissue during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
AA	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Alanine																	
PDV[5]	2.09	0.20	—	—	—	—	—	—	—	—	—	—	0.64	0.46	0.56	0.53	0.11
Hepatic	—	—	−1.74	0.54	−2.33	0.54	−3.09	0.54	−2.85	0.54	−3.50	0.54	0.20	0.16	0.16	0.65	0.02
Splanchnic	−0.60	0.33	—	—	—	—	—	—	—	—	—	—	0.62	0.83	0.83	0.76	0.44
Aspartate																	
PDV	—	—	0.07	0.21	0.72	0.21	0.61	0.21	0.54	0.21	1.19	0.21	0.46	0.005	0.56	0.96	0.002
Hepatic	−0.25	0.09	—	—	—	—	—	—	—	—	—	—	0.28	0.17	0.90	0.18	0.13
Splanchnic	0.37	0.14	—	—	—	—	—	—	—	—	—	—	0.90	0.12	0.82	0.30	0.23
Glutamate	—	—															
PDV	−0.43	0.20	−0.82	0.18	−0.46	0.18	−0.49	0.18	0.09	0.18	0.27	0.18	0.42	<0.001	0.75	0.66	<0.001
Hepatic	1.50	0.10	—	—	—	—	—	—	—	—	—	—	0.11	0.61	0.52	0.35	0.96
Splanchnic	—	—	0.84	0.23	0.86	0.23	1.14	0.23	1.36	0.23	1.85	0.23	0.59	0.003	0.35	0.23	<0.001
Glutamine																	
PDV	—	—	−9.27	0.68	−6.09	0.68	−5.56	0.68	−3.41	0.68	−2.80	0.68	0.96	<0.001	0.73	0.13	<0.001
Hepatic	—	—	1.16	0.65	−1.13	0.65	−2.20	0.65	−4.41	0.65	−4.32	0.65	0.38	<0.001	0.40	0.08	<0.001
Splanchnic	−7.50	0.31	—	—	—	—	—	—	—	—	—	—	0.66	0.77	0.85	0.50	0.77
Glycine																	
PDV	2.00	0.25	—	—	—	—	—	—	—	—	—	—	0.48	0.81	0.14	0.70	0.74
Hepatic	—	—	0.07	0.66	0.06	0.66	−1.84	0.66	−1.75	0.66	−0.14	0.66	0.22	0.07	0.09	0.03	0.28
Splanchnic	1.19	0.36	—	—	—	—	—	—	—	—	—	—	0.40	0.17	0.45	0.14	0.92
Proline																	
PDV	—	—	0.37	0.17	0.63	0.17	0.89	0.17	0.92	0.17	1.34	0.17	0.47	0.002	0.11	0.88	<0.001
Hepatic	—	—	−0.18	0.17	−0.19	0.17	−0.79	0.17	−0.81	0.17	−0.84	0.17	0.22	0.003	0.13	0.32	0.001
Splanchnic	0.26	0.09	—	—	—	—	—	—	—	—	—	—	0.59	0.52	0.29	0.63	0.48
Serine																	
PDV	—	—	0.99	0.22	1.29	0.22	1.55	0.22	1.73	0.22	2.13	0.22	0.20	0.006	0.18	0.89	<0.001
Hepatic	—	—	−0.50	0.21	−0.85	0.21	−1.37	0.21	−1.36	0.22	−1.55	0.22	0.02	0.005	0.08	0.30	0.001
Control	−1.38	0.14	—	—	—	—	—	—	—	—	—	—					
Glucose	−0.87	0.13	—	—	—	—	—	—	—	—	—	—					
Splanchnic													0.39	0.76	0.02	I = 0.003	0.76
Control	—	—	−0.08	0.31	0.77	0.31	0.35	0.31	0.30	0.32	0.13	0.31					
Glucose	—	—	0.91	0.30	0.16	0.30	−0.003	0.30	0.56	0.30	0.93	0.30					
Tyrosine																	
PDV	—	—	0.57	0.15	0.73	0.15	0.92	0.15	0.80	0.15	0.99	0.15	0.26	0.28	0.18	0.66	0.05
Hepatic	—	—	−0.57	0.15	−0.48	0.15	−0.96	0.15	−0.88	0.15	−0.83	0.15	0.13	0.11	0.68	0.37	0.05
Splanchnic	0.04	0.07	—	—	—	—	—	—	—	—	—	—	0.73	0.66	0.58	0.93	0.90

[1]Treatments consisted of abomasal glucose infusion of 3.84 g/h (glucose) or no glucose infusion (control) and 5 levels of abomasal protein infusion that consisted of a soy protein and cysteine in a ratio of 0.09 g of cysteine per gram of soy protein.

[2]Overall algebraic mean and SE for all observations n = 90 for PDV and 85 for hepatic and splanchnic unless otherwise noted. Treatment means are least squares means and SE.

[3]ANOVA was analyzed as a mixed model with glucose treatment (glucose), protein infusion level (protein), the interaction of glucose and protein (G × P), and period as fixed effects and animal with treatment as a random effect. Nine animals each were in the control and glucose treatment. Eight observations were observed for hepatic and splanchnic release in the control treatment.

[4]Probability estimates for the quadratic and linear orthogonal contrast for protein infusion. The values labeled as equal to I are the *P*-values associated with interaction between protein and glucose treatment when the interaction was significant.

[5]Portal-drained viscera.

Table 4. Hepatic extraction: net hepatic AA uptake (mmol/h) = $f(x) = b_2x^2 + b_1x + b_0$, where x = AA delivery rate to the liver (mmol/h)

AA	Range delivered,[1] mmol/h	b_2	±SE	b_1	±SE	b_0	±SE	$P_{Glucose}$[2]	$P_{Quadratic}$[3]	P_{Linear}[3]
Essential										
Histidine	0.2 to 32.3	0.02216	0.00795	−0.2579	0.2513	0.5496	1.0432	0.23	0.007	0.31
Lysine	2.1 to 34.7							0.07	—	I = 0.006
Control		—	—	0.1477	0.0556	−1.6361	0.7095			
Glucose		—	—	0.2399	0.0479	−1.6361	0.7095			
Phenylalanine	2.7 to 14.8							0.06	I = 0.02	I = 0.02
Control		−0.01138	0.00576	0.3207	0.1012	−0.7019	0.4319			
Glucose		−0.00519	0.00683	0.2488	0.1100	−0.7019	0.4319			
Valine	6.5 to 57.8							0.14	I = 0.03	I = 0.05
Control		0.00243	0.00116	−0.0704	0.0724	0.8333	1.0284			
Glucose		0.00499	0.00188	−0.0149	0.0901	0.8333	1.0284			
Isoleucine	3.5 to 16.1	—	—	0.0643	0.0246	−0.4008	0.2677	1.00	—	0.01
Leucine	5.8 to 26.0	—	—	0.0600	0.0245	−0.5957	0.4215	1.00	—	0.02
Methionine	0.7 to 3.5	—	—	0.1136	0.0246	−0.0868	0.0549	0.78	—	<0.001
Threonine	3.0 to 28.2	—	—	0.0579	0.0252	−0.2197	0.3980	0.18	—	0.10
Nonessential										
Alanine	10.2 to 38.5	—	—	0.1502	0.0438	−1.1893	1.0004	0.69	—	<0.001
Aspartate	0.4 to 8.3	—	—	0.1537	0.0488	0.0756	0.2384	0.26	—	0.004
Glutamine	23.5 to 108.3	—	—	0.07784	0.0238	3.7804	2.0396	0.70	—	0.003
Glycine	17.0 to 55.8	—	—	0.07955	0.04088	−1.8288	1.4160	0.38	—	0.02
Proline	4.2 to 18.4	—	—	0.1033	0.02311	−0.6856	0.2952	0.74	—	<0.001
Serine	3.9 to 17.0	—	—	0.1697	0.0324	−0.7115	0.3659	0.27	—	<0.001
Tyrosine	3.1 to 17.8	—	—	0.09960	0.0216	−0.1777	0.2143	0.55	—	<0.001

[1]Regressions were conducted on all observations for all ewes (control n = 40 and glucose n = 45 except control Lys, and His n = 37 and glucose Lys n = 43 and His n = 42).

[2]Probability that glucose-infused ewes differed from control ewes.

[3]Probability that the linear and quadratic regression coefficient differed from zero. The values labeled as equal to I are the *P*-values associated with interaction between protein and glucose treatment when the interaction was significant.

The experimental design was a split-plot with a complete randomized design on the whole-plot (glucose infusion) and a Latin-square subplot with 5 periods and incremental levels of protein infusion. The data were analyzed with a model that accounted for period, glucose infusion, protein infusion level, and the interaction between glucose infusion and protein infusion level as fixed effects, and animal within glucose level as a random effect using the MIXED procedure (SAS Inst. Inc. Cary, NC). Glucose infusion levels were tested using the whole-plot error term (animal within glucose infusion), and protein infusion level and the interaction between glucose infusion and protein infusion level were tested with the subplot error term (residual). Linear and quadratic effects of protein infusion level were tested with orthogonal contrasts. Hepatic extraction ratio was calculated across all animals by regressing net hepatic uptake on delivery rate (arterial concentration × arterial blood flow + portal concentration × portal blood flow). Linear and quadratic responses for hepatic extraction were tested with the same design except AA delivery rate was treated as a linear covariate, quadratic covariate, or both. A step-down procedure was used to determine whether hepatic excretion response was quadratic or linear. Statistical analyses were conducted using the MIXED procedure in SAS. Means were considered statistically different when $P < 0.05$, and means were considered to tend to differ when $0.05 < P < 0.10$.

RESULTS

The hepatic catheter failed in one of the control ewes, resulting in missed observations for hepatic concentrations, on metabolites and net release of hepatic and splanchnic metabolites.

PDV

Portal venous blood flow decreased linearly with protein infusion ($P = 0.04$; Table 1). Portal-drained viscera oxygen consumption did not differ between ewes receiving glucose (148.4 ± 6.5 mmol/h) and control ewes (148.8 ± 6.2 mmol/h; $P = 0.89$; Table 1).

Control ewes had a negative release (net uptake) of glucose (Table 1). Net PDV glucose release was greater ($P < 0.001$) and was positive in ewes abomasally infused with glucose (Table 1). Net glucose release from the PDV responded quadratically to protein infusion ($P = 0.03$) with net release being greater at intermediate levels of protein infusion (Table 1). Net PDV lactate release from glucose-infused ewes (7.33 ± 0.78 mmol/h) did not differ ($P = 0.33$) from control ewes (8.45 ± 0.78 mmol/h) and did not differ linearly ($P = 0.16$) or quadratically ($P = 0.40$) with protein infusion.

Net PDV ammonia release did not differ ($P = 0.42$) between glucose-infused ewes (30.5 ± 1.79 mmol/h) and control ewes (32.6 ± 1.79 mmol/h) and did not

Table 5. Blood concentration of essential AA (n*M*) during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
AA	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Histidine[5]																	
Arterial	98.9	5	—	—	—	—	—	—	—	—	—	—	0.70	0.45	0.75	0.31	0.60
Portal	51.8	3	—	—	—	—	—	—	—	—	—	—	0.14	0.57	0.43	0.21	0.60
Hepatic	58.5	4	—	—	—	—	—	—	—	—	—	—	0.08	0.92	0.40	0.59	0.83
Isoleucine																	
Arterial													0.01	<0.001	0.10	0.67	I = 0.04
Control	—	—	61.4	4.3	59.4	4.3	71.1	4.3	76.4	4.3	80.0	4.3					
Glucose	—	—	37.9	4.3	51.4	4.3	58.0	4.3	67.3	4.3	73.1	4.3					
Portal	—	—											0.02	<0.001	0.19	0.71	I = 0.04
Control	—	—	66.5	5.0	67.2	5.0	78.2	5.0	87.2	5.0	90.8	5.0					
Glucose	—	—	41.6	5.0	56.0	5.0	68.3	5.0	74.4	5.0	85.8	5.0					
Hepatic	—	—	52.9	3.6	61.0	3.6	68.4	3.6	78.1	3.6	85.0	3.6	0.02	<0.001	0.40	1.00	<0.001
Leucine																	
Arterial	—	—	84.0	5.3	88.5	5.3	103.0	5.3	116.3	5.3	124.9	5.3	0.055	<0.001	0.32	0.62	<0.001
Control	111.7	5.7	—	—	—	—	—	—	—	—	—	—					
Glucose	95.0	5.7	—	—	—	—	—	—	—	—	—	—					
Portal	—	—	90.1	5.9	97.4	5.9	115.2	5.9	129.5	5.9	142.5	5.9	0.06	<0.001	0.48	0.64	<0.001
Control	123.8	6.2	—	—	—	—	—	—	—	—	—	—					
Glucose	106.1	6.2	—	—	—	—	—	—	—	—	—	—					
Hepatic	—	—	89.4	6.0	96.8	5.9	108.7	5.9	125.7	6.0	138.0	5.9	0.045	<0.001	0.69	0.37	<0.001
Control	121.4	6.4	—	—	—	—	—	—	—	—	—	—					
Glucose	102.0	6.1	—	—	—	—	—	—	—	—	—	—					
Lysine[6]																	
Arterial	—	—	77.0	8.3	86.0	8.1	87.3	8.3	98.1	8.3	103.1	8.6	0.30	0.18	0.22	0.96	0.02
Portal	—	—	86.0	10.8	93.4	10.5	113.2	10.8	113.2	10.8	123.5	11.1	0.88	0.10	0.27	0.73	0.008
Hepatic	98.9	4.8	92.7	9.3	92.5	9.0	95.9	9.3	108.4	9.3	110.8	10.0	0.41	0.47	0.63	0.68	0.09
Methionine																	
Arterial													0.046	0.02	0.12	0.72	I = 0.01
Control	—	—	13.4	0.9	12.7	0.9	13.2	0.9	13.8	0.9	13.5	0.9					
Glucose	—	—	9.3	0.9	10.6	0.9	11.7	0.9	12.7	0.9	13.0	0.9					
Portal													0.04	<0.001	0.06	0.95	I = 0.01
Control	16.1	0.7	15.7	1.0	15.3	1.0	15.4	1.0	16.9	1.0	17.3	1.0					
Glucose	13.8	0.7	10.8	1.0	12.3	1.0	14.6	1.0	15.0	1.0	16.2	1.0					
Hepatic													0.07	0.009	0.19	0.77	I = 0.03
Control	14.4	0.8	12.3	0.7	12.8	0.7	13.1	0.7	14.3	0.7	14.6	0.7					
Glucose	12.4	1.0	14.2	1.0	14.2	1.0	13.6	1.0	15.1	1.0	15.0	1.0					
Phenylalanine																	
Arterial	—	—	37.1	2.3	40.5	2.3	48.9	2.3	56.9	2.3	62.9	2.3	0.22	<0.001	0.61	0.54	<0.01
Portal	—	—	42.3	2.9	48.3	2.9	58.6	2.9	67.4	2.9	76.4	2.9	0.19	<0.001	0.74	0.65	<0.01
Hepatic	—	—	36.7	2.7	42.2	2.7	48.6	2.7	58.0	2.7	65.8	2.7	0.17	<0.001	0.96	0.40	<0.01
Threonine																	
Arterial	—	—	82.1	6.6	86.2	6.6	98.0	6.6	101.7	6.6	108.4	6.6	0.02	0.002	0.40	0.88	<0.01
Control	108.4	7.0	—	—	—	—	—	—	—	—	—	—					
Glucose	82.1	7.0	—	—	—	—	—	—	—	—	—	—					

Continued

Table 5 (Continued). Blood concentration of essential AA (n*M*) during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
AA	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Portal	—	—	85.4	6.7	92.1	6.7	104.9	6.7	109.7	6.7	118.2	6.7	0.01	<0.001	0.64	0.81	<0.01
Control	115.9	6.8	—	—	—	—	—	—	—	—	—	—					
Glucose	88.3	6.8	—	—	—	—	—	—	—	—	—	—					
Hepatic	—	—	81.1	6.9	90.2	6.9	96.0	6.9	105.9	6.9	110.3	6.9	0.04	0.001	0.75	0.79	<0.01
Control	108.5	7.4	—	—	—	—	—	—	—	—	—	—					
Glucose	84.9	7.0	—	—	—	—	—	—	—	—	—	—					
Valine																	
Arterial													0.04	0.02	0.22	0.10	0.006[7]
Control	183.5	10.1	176.5	18.1	157.8	18.1	213.1	18.1	188.8	18.1	181.3	18.1					
Glucose	151.7	10.1	104.8	18.1	141.0	18.1	159.8	18.1	182.2	18.1	170.7	18.1					
Portal													0.04	0.01	0.32	0.11	0.003[7]
Control	192.7	10.2	182.6	19.0	168.7	19.0	218.3	19.0	202.4	19.0	191.4	19.0					
Glucose	159.7	10.2	107.0	19.0	145.8	19.0	173.2	19.0	187.8	19.0	185.0	19.0					
Hepatic	—	—	144.1	13.0	158.2	13.0	186.1	13.0	191.6	13.0	185.7	13.0	0.03	0.03	0.39	0.18	0.004
Control	189.7	10.2	—	—	—	—	—	—	—	—	—	—					
Glucose	156.6	9.6	—	—	—	—	—	—	—	—	—	—					

[1]Treatments consisted of abomasal glucose infusion of 3.84 g/h (glucose) or no glucose infusion (control) and 5 levels of abomasal protein infusion that consisted of a soy protein and cysteine ratio of 0.09 g of cysteine per gram of soy protein.

[2]Overall algebraic mean and SE for all observations n = 90 for portal-drained viscera and 85 for hepatic and splanchnic unless otherwise noted. Treatment means are least squares means and SE.

[3]ANOVA was analyzed as a mixed model with glucose treatment (glucose), protein infusion level (protein), the interaction of glucose and protein (G × P), and period as fixed effects and animal with treatment as a random effect. Nine animals each were in the control and glucose treatment. Eight observations were observed for hepatic concentration in the control treatment.

[4]Probability estimates for the quadratic and linear orthogonal contrast for protein infusion. The values labeled as equal to I are the *P*-values associated with interaction between protein and glucose treatment when the interaction was significant.

[5]Missing observations for 1 control animal at protein infusion levels 0, 5.2, and 10.5 g/h and 2 glucose animals at 7.8 g/h and 1 glucose animal at 10.5 g/h.

[6]Missing observation for 1 control animal at protein infusion levels 0, 5.2, and 10.5 g/h and 1 glucose animal at 7.8 and 10.5 g/h.

[7]Interaction between protein and glucose treatment tended to differ ($0.05 < P < 0.10$).

Table 6. Blood concentration of nonessential AA (n*M*) during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
AA	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Alanine																	
Arterial													0.19	0.09	0.04	0.69	I = 0.005
Control	—	—	165.4	10.8	152.8	10.8	154.9	10.8	164.4	10.8	159.3	10.8					
Glucose	—	—	119.3	10.8	138.0	10.8	146.7	10.8	150.3	10.8	169.6	10.8					
Portal													0.19	0.003	0.009	0.34	I = 0.001
Control	—	—	182.7	11.3	168.0	11.3	169.2	11.3	184.9	11.3	180.5	11.3					
Glucose	—	—	131.7	11.3	145.8	11.3	165.7	11.3	169.4	11.3	169.9	11.3					
Hepatic													0.57	0.12	0.04	0.26	I = 0.002
Control	—	—	158.7	11.2	142.8	11.2	143.4	11.2	154.2	11.2	144.6	11.2					
Glucose	—	—	125.4	10.5	130.8	10.5	137.0	10.5	152.1	10.5	167.4	10.5					
Aspartate																	
Arterial	—	—	10.7	2.3	13.8	2.3	18.4	2.3	20.1	2.3	24.1	2.3	0.15	0.002	0.69	0.87	<0.001
Portal	—	—	11.4	2.6	19.9	2.6	23.7	2.6	25.5	2.6	35.0	2.6	0.08	<0.001	0.72	0.98	<0.001
Hepatic	—	—	11.4	2.6	19.8	2.6	19.6	2.6	22.6	2.6	31.4	2.6	0.09	<0.001	0.84	0.67	<0.001
Glutamate																	
Arterial													0.13	<0.001	0.04	0.16	I = 0.005
Control	—	—	45.4	2.5	41.0	2.5	46.0	2.5	50.8	2.5	51.2	2.5					
Glucose	—	—	34.3	2.5	37.6	2.5	43.4	2.5	46.9	2.5	54.9	2.5					
Portal													0.12	<0.001	0.06	0.13	I = 0.01
Control	—	—	39.4	3.1	38.7	3.1	41.8	3.1	52.8	3.1	53.7	3.1					
Glucose	—	—	27.4	3.1	32.0	3.1	40.0	3.1	46.8	3.1	57.3	3.1					
Hepatic													0.51	<0.001	0.11	0.04	I = 0.04
Control	—	—	48.7	3.8	48.6	3.8	54.1	3.8	63.8	3.9	65.6	3.8					
Glucose	—	—	42.0	3.6	42.7	3.6	52.3	3.6	58.1	3.6	73.0	3.6					
Glutamine																	
Arterial													0.40	0.10	0.02	0.28	I = 0.003
Control	—	—	523.8	28.6	458.7	28.6	524.9	28.6	504.5	28.6	465.6	28.6					
Glucose	—	—	407.6	28.6	449.1	28.6	497.7	28.6	492.8	28.6	510.4	28.6					
Portal													0.39	<0.001	0.05	0.08	I = 0.006
Control	—	—	441.0	26.5	410.7	26.5	478.2	26.5	474.1	26.5	448.0	26.5					
Glucose	—	—	341.7	26.5	394.5	26.5	455.0	26.5	464.7	26.5	477.5	26.5					
Hepatic													0.62	0.02	0.12	0.13	I = 0.03
Control	—	—	429.6	26.7	406.4	26.7	458.6	26.7	455.3	26.9	414.6	26.7					
Glucose	—	—	363.4	25.2	398.1	25.2	443.7	25.2	433.7	25.2	459.7	25.2					
Glycine																	
Arterial	230.4	5.1	—	—	—	—	—	—	—	—	—	—	0.64	0.31	0.16	0.52	0.13
Portal	247.2	5.8	243.2	11.4	234.9	11.4	241.0	11.4	255.9	11.4	260.9	11.4	0.51	0.35	0.09	0.36	0.09
Hepatic	—	—	229.4	10.2	232.4	10.2	226.6	10.2	241.9	10.2	256.2	10.2	0.87	0.14	0.91	0.20	0.03
Proline																	
Arterial													0.11	<0.001	0.13	0.87	I = 0.04
Control	—	—	75.7	5.1	74.5	5.1	84.1	5.1	92.0	5.1	97.0	5.1					
Glucose	—	—	54.3	5.1	68.3	5.1	76.4	5.1	83.3	5.1	94.2	5.1					

Continued

Table 6 (Continued). Blood concentration of nonessential AA (n*M*) during abomasal protein infusion with and without glucose[1]

	Overall/ treatment[2]		Least squares means and SE for abomasal protein infusion level, g/h										*P*-value for ANOVA[3]			Contrast *P*-value[4]	
AA	Mean	±SE	0	±SE	2.6	±SE	5.2	±SE	7.8	±SE	10.5	±SE	Glucose	Protein	G × P	Quadratic	Linear
Portal																	
Control	—	—	79.3	5.6	81.6	5.6	90.2	5.6	102.1	5.6	107.5	5.6	0.10	<0.001	0.09	0.69	I = 0.02
Glucose	—	—	56.3	5.6	71.1	5.6	84.6	5.6	89.7	5.6	108.8	5.6					
Hepatic	—	—	66.2	4.0	74.6	4.0	80.4	4.0	90.2	4.0	100.5	4.0	0.15	<0.001	0.45	0.48	<0.001
Serine																	
Arterial													0.03	<0.001	0.08	0.69	I = 0.02
Control	—	—	69.0	5.1	61.2	5.1	73.8	5.1	73.6	5.1	74.9	5.1					
Glucose	—	—	43.0	5.1	54.2	5.1	59.6	5.1	68.2	5.1	70.4	5.1					
Portal													0.02	<0.001	0.20	0.94	I = 0.02
Control	—	—	77.8	5.9	75.1	5.9	85.3	5.9	91.5	5.9	92.8	5.9					
Glucose	—	—	50.0	5.9	61.0	5.9	73.6	5.9	80.8	5.9	91.4	5.9					
Hepatic													0.07	<0.001	0.33	0.97	<0.001[5]
Control	—	—	67.0	5.5	65.1	5.5	74.5	5.5	79.8	5.6	77.5	5.5					
Glucose	—	—	48.8	5.2	55.8	5.2	60.1	5.2	72.5	5.2	77.6	5.2					
Tyrosine																	
Arterial	—	—	46.5	3.6	49.0	3.6	56.5	3.6	66.4	3.6	68.4	3.6	0.37	<0.001	0.75	0.88	<0.001
Portal	—	—	51.0	4.0	54.8	4.0	63.9	4.0	73.4	4.0	77.6	4.0	0.31	<0.001	0.75	0.92	<0.001
Hepatic	—	—	46.9	3.7	51.2	3.7	56.9	3.7	68.4	3.7	71.0	3.7	0.25	<0.001	0.96	0.82	<0.001

[1]Treatments consisted of abomasal glucose infusion of 3.84 g/h (glucose) or no glucose infusion (control) and 5 levels of abomasal protein infusion that consisted of a soy protein and cysteine ratio of 0.09 g of cysteine per gram of soy protein.

[2]Overall algebraic mean and SE for all observations n = 90 for portal-drained viscera and 85 for hepatic and splanchnic unless otherwise noted. Treatment means are least squares means and SE.

[3]ANOVA was analyzed as a mixed model with glucose treatment (glucose), protein infusion level (protein), the interaction of glucose and protein (G × P), and period as fixed effects and animal with treatment as a random effect. Nine animals each were in the control and glucose treatment. Eight observations were observed for hepatic and splanchnic release in the control treatment.

[4]Probability estimates for the quadratic and linear orthogonal contrast for protein infusion. The values labeled as equal to I are the *P*-values associated with interaction between protein and glucose treatment when the interaction was significant.

[5]Interaction between protein and glucose treatment tended to differ ($0.05 < P < 0.10$).

differ linearly ($P = 0.70$) or quadratically ($P = 0.49$) with protein infusion. There was a negative release (net uptake) of urea by the PDV, and a glucose infusion × protein infusion interaction (linear; $P = 0.008$; Table 1).

Net PDV release of isoleucine, leucine, methionine, and phenylalanine increased linearly with increased protein infusion ($P < 0.001$ to 0.02), and threonine tended to increase linearly ($P = 0.09$; Table 2). Net PDV release of aspartate, glutamate, glutamine, proline, serine, and tyrosine increased linearly ($P < 0.001$ to 0.05; Table 3) with increased protein infusion; however, glutamine release remained negative (net uptake) over the range of infusion levels. Net release of glutamate was negative initially and became less negative as protein infusion increased (Table 3). There was a net PDV release of alanine, glycine, and aspartate (Table 3). Net release of individual nonessential AA did not differ ($P = 0.20$ to 0.64) between glucose-infused and control ewes (Table 3).

Liver

Hepatic arterial blood flow responded quadratically to increased protein infusion; intermediate levels of protein infusion have decreased blood flows ($P = 0.003$; Table 1). There was an interaction between glucose infusion and the linear relationship of protein infusion ($P = 0.005$) for hepatic venous blood flow ($P = 0.005$; Table 1), where initial rates of blood flow were greater for the glucose-infused ewes. Hepatic oxygen consumption responded quadratically to increased protein infusion with intermediate levels of protein infusion having decreased rates of oxygen consumption ($P = 0.05$; Table 1).

Net hepatic glucose release was less in glucose-infused ewes (Table 1; $P = 0.007$) than in control ewes and increased quadratically with protein infusion in both treatments (Table 1; $P = 0.004$). Net hepatic lactate release was negative (net uptake), and ewes infused with glucose (-6.96 ± 1.33 mmol/h) did not differ from control ewes ($P = 0.07$; -3.22 ± 1.33 mmol/h) nor did it differ linearly ($P = 0.80$) or quadratically $P = 0.56$) with protein infusion.

Net hepatic ammonia release was negative (net uptake), and ewes infused with glucose (-31.59 ± 1.75 mmol/h) did not differ from control ewes ($P = 0.58$; -33.03 ± 1.86 mmol/h) nor did they differ linearly ($P = 0.86$) or quadratically with ($P = 0.27$) with protein infusion. Net hepatic urea release increased linearly with increased protein infusion ($P < 0.001$; Table 1).

Net hepatic release of threonine was negative for both treatments, and net release was greater in glucose-infused ewes than control ewes ($P = 0.04$; Table 2). Net hepatic release of lysine, methionine, and phenylalanine decreased (increased uptake) with increased protein infusion ($P = 0.001$ to 0.006), and net hepatic threonine tended to decrease ($P = 0.07$; Table 2). Net hepatic histidine responded quadratically with protein infusion, and there was an interaction between protein infusion and glucose treatment ($P = 0.04$; Table 2). Net hepatic release of glutamine decreased linearly with increased protein infusion such that the release rate became negative ($P < 0.001$; Table 3). Net hepatic release of alanine, proline, tyrosine, and serine was negative (net uptake) and decreased linearly with increased protein infusion ($P = 0.001$ to 0.05; Table 3), and net serine release was greater in glucose-infused ewes compared with control ewes ($P = 0.02$; Table 3). There was a net hepatic release of glutamate (Table 3).

With the exception of histidine, phenylalanine, and valine, net hepatic AA uptake increased linearly with increased delivery of AA (Table 4). Glucose infusion increased the hepatic uptake of lysine and valine with respect to delivery rate, and phenylalanine extraction decreased (Table 4). Hepatic histidine uptake decreased quadratically with respect to delivery rate (Table 4).

Blood Metabolites

There was an interaction between glucose infusion treatment and protein infusion ($P < 0.001$; Table 1). Arterial concentrations of blood glucose increased with glucose infusion and increased linearly with increased protein infusion ($P < 0.001$; Table 1). Portal vein glucose concentrations followed a similar pattern as arterial glucose concentrations (Table 1). The pattern of hepatic glucose concentrations differed from that of arterial and portal in that the glucose concentration of glucose-infused ewes was not greater than control ewes (Table 1).

Arterial and portal vein concentrations of urea-N did not differ with glucose infusion, and the concentration of both increased linearly with protein infusion (Table 1). Hepatic urea-N tended to decrease ($P = 0.09$) with glucose infusion and increased linearly ($P = 0.005$; Table 1) with protein infusions.

With the exception of histidine, circulating concentrations of essential AA increased linearly with increased protein infusion ($P \leq 0.001$ to 0.02; Table 5). Arterial isoleucine concentrations were greater in control ewes but increased at a slower rate with protein infusion compared with glucose-infused ewes ($P = 0.04$; Table 5). Arterial concentrations of leucine, methionine, threonine, and valine increased ($P < 0.02$ to 0.055) with glucose infusion (Table 5).

With the exception of glycine ($P = 0.13$), arterial concentrations of nonessential AA increased with increased protein infusion (Table 6). The rate of increase differed between treatments for alanine, glutamate, glutamine, proline, and serine (Table 6).

DISCUSSION

A short-term infusion was selected over a long-term infusion. Responses to the 2 experimental models may differ to the extent that long-term infusion would allow the intestinal tissue to adapt and use protein as

an energy source when it is in excess. Feed intake levels were set to provide approximately the amount of ME required for maintenance, and CP was set slightly greater than maintenance for nonpregnant, nonlactating ewes (NRC, 2007). Protein infusion rates were set such that the intermediate infusion rate would be similar to the endogenous flow rate. Glucose infusion rate was set equal to the predicted net hepatic release of glucose in nonpregnant, nonlactating ewes (Freetly and Klindt, 1996). Our previous study (Freetly and Klindt, 1996), demonstrated a quick adaptation by the liver to increased glucose entry rate.

Amino acids that enter the intestinal epithelium are used for protein synthesis (structural and secretory), catabolized for energy, or released into the blood through the basal membrane. Studies in sheep (MacRae et al., 1997) and in pigs (Stoll et al., 1998) reported that approximately one-third of the AA absorbed by the enterocyte are metabolized within the enterocyte and are never released into the blood. Of those AA that are metabolized within the enterocyte, 60% are most likely catabolized (Stoll et al., 1998), which suggests that 20% of the absorbed AA are catabolized and used as a source of energy within the enterocyte. Reducing the proportion of AA catabolized for energy could potentially result in an increased efficiency of dietary AA being released into the blood. Enteral glucose oxidation increased in the neonatal pig when dietary protein was decreased (van der Schoor et al., 2001). Our hypothesis was that catabolism of AA would be reduced by increasing the supply of glucose as an alternative energy substrate. In ruminants, little glucose escapes rumen fermentation, particularly on forage diets. Some glucose is available for absorption from the lumen in diets that have a large quantity of bypass starch (Kreikemeier et al., 1991; Bauer et al., 1995). In the current study, ewes that did not receive glucose infusion had a net uptake of glucose by the PDV, suggesting that glucose was being removed from arterial blood and catabolized by PDV tissue. Net PDV release of glucose increased 14.5 mmol/h compared with control ewes, which resulted in a net release of glucose from the PDV. This increase in release represented 68% of the glucose infused into the abomasum (21.3 mmol/h). In a study with steers, Kreikemeier et al. (1991) reported that net PDV glucose release increased with increased abomasal glucose infusion and that the increase in net PDV release accounted for 62 to 108% of the infused glucose. There are tissues within the PDV that are net users of glucose (i.e., rumen complex and mesenteric adipose). The ratio of net release to infusion rate is an underestimate of net appearance across the small intestine. Kreikemeier et al. (1991) reported that disappearance rates from the lumen ranged from 71 to 100%. The positive net PDV release of glucose with glucose infusion suggests that glucose absorbed from the lumen was in excess of that metabolized by the mucosal cells. Studies in neonatal pigs (Reeds et al., 2000) indicated that catabolism of enteral glucose is incomplete with 68% of the carbon appearing as lactate and alanine. In the current study, PDV lactate and alanine fluxes did not increase with glucose infusion.

There were no differences in net PDV release of individual AA when glucose was infused, suggesting that glucose did not spare AA from catabolism in the mucosa. For most AA, there was a net PDV release of AA with the notable exceptions being glutamate and glutamine. Tracer studies in the neonatal pig (Reeds et al., 2000) have shown that over 95% of the dietary glutamate and 11% of the glutamate arriving from the arterial blood are extracted by the PDV. Those data indicate that 63% of the dietary glutamate carbon could be accounted for in products of catabolism (CO_2, lactate, alanine). In the neonatal pig, 36% of the CO_2 originates from glutamate catabolism (Reeds et al., 2000). In addition to catabolism, glutamate is used for biosynthetic purposes including protein synthesis and as a precursor for glutathione, arginine, and proline (Reeds et al., 2000). In this study, there was a net uptake of glutamate until protein infusion rate reached 10.5 g/h, and then there was a net release. Our findings suggest that it is possible to saturate the capacity of the PDV to catabolize and metabolize glutamate.

Results from reports on net flux of glutamine from PDV have been mixed with some studies reporting a net uptake (Heitmann and Bergman, 1981; Reeds et al., 2000; current study), and others have reported a net release (Lobley et al., 2003; Hanigan et al., 2004b). Isotope studies in neonatal pigs (Reeds et al., 2000) reported elevated (22%) PDV extraction of glutamine from arterial blood and that arterial glutamine accounted for 15% of the CO_2. In the current study, there was a net negative PDV release of glutamine, but the rate of release increased with increased protein infusion rates. Doepel et al. (2007) reported an increased net release of glutamine when glutamine was infused into the abomasum of dairy cows and that 83% of the infused glutamine could be accounted for by the increase in net PDV glutamine and glutamate release. Data from our study, combined with the observations of Doepel et al. (2007), suggest that although PDV metabolizes glutamine, absorption of dietary glutamine can exceed the rate of metabolism.

Net PDV release of isoleucine, leucine, methionine, phenylalanine, aspartate proline, serine, and tyrosine all increased linearly with increased abomasal protein infusion. There were no quadratic effects, suggesting that the capacity for absorption and transport of these AA into the circulation were not limiting. El-Kadi et al. (2006) observed linear increases in net release of these same AA when casein was infused into the duodenum of sheep at a rate of 105 g/d with the exception of serine. Net PDV release of alanine, glycine, histidine, threonine, and valine remained constant across protein infusion levels, which were in contrast to the linear increases observed by El-Kadi et al. (2006).

Net hepatic glucose release decreased 5.5 mmol/h with glucose infusion, suggesting that hepatic gluco-

neogenesis is decreased with increased PDV release of glucose. These observations are consistent with our earlier observations that reported a decrease in net hepatic glucose release when glucose was infused into the mesenteric venous drainage (Freetly and Klindt, 1996). In the current study, net hepatic glucose release increased with increased protein infusion. In nonpregnant, nonlactating ewes, propionate and AA accounted for the majority of the carbon taken up by the liver (Freetly and Ferrell, 2000). The increase in net hepatic glucose release with abomasal protein infusion was accompanied by an increase in net hepatic uptake of gluconeogeneic AA (alanine, glutamine, glycine, histidine, methionine, proline, serine, and tyrosine). The increase occurred in the control and glucose-infused ewes, suggesting that increasing gluconeogeneic AA to the liver "pushed" gluconeogenesis even when there appeared to be feedback inhibition on the liver. Wray-Cahen et al. (1997) also observed an increase in net hepatic glucose flux when AA were infused into the mesenteric vein of cattle.

In the Hanigan et al. (2004a) model of AA uptake by the liver of lactating cows, AA that were taken up by the liver were modeled using mass action kinetics. In our study, hepatic uptake of most of the AA was linearly related to delivery rate, suggesting that for most AA, mass action kinetics adequately described AA uptake by the liver of the ewe. Lobley et al. (2001) infused AA directly into the mesenteric vein of sheep and found linear relations between net hepatic uptakes and delivery rates for most AA in whole blood except isoleucine, leucine, proline, valine, citrulline, and ornithine. Those were neither linearly nor quadratically related to delivery rate. Exceptions in our study were histidine, phenylalanine, and valine. Histidine had a quadratic response where net uptake was insensitive to delivery rate until approximately 10 mmol/h after which hepatic extraction could be described by a linear function. There was a quadratic response for net hepatic valine release. A linear response would have adequately described the relationship between valine uptake and delivery rate in control ewes; however, in glucose-infused ewes, the rate of hepatic valine uptake increased with delivery rate. In control ewes, net hepatic phenylalanine release increased until delivery rate reached 11.6 mmol/h. These observations suggest that phenylalanine release is regulated. Phenylalanine release did not change in glucose-infused ewes, suggesting that the capacity of the liver to remove phenylalanine from blood had not been exceeded.

We observed a net hepatic release of glutamate. Heitmann and Bergman (1981) reported the majority of the glutamate being released from the liver was from nonglutamine sources and was presumably being synthesized from α-ketoglutarate. Besides being a principal energy source for the PDV, there is a net uptake of glutamate by the hindquarters of sheep (Heitmann and Bergman, 1981), suggesting that hepatic release is supporting a net uptake by PDV and peripheral tissue. In that study, conversion of glutamate to glutamine was relatively low in PDV and hindquarters.

Based on the observations in the current study, we reject our hypothesis that glucose can spare AA metabolism by PDV tissue. Our findings suggest that hepatic gluconeogenesis can be increased in the presence of increased AA delivery to the liver and that hepatic gluconeogenesis can be decreased with increased absorption of dietary glucose. Our findings support the concept that for most AA, hepatic uptake of AA can be described by mass action kinetics. However, the rates of hepatic uptake of specific amino are upregulated directly or indirectly by elevated glucose.

LITERATURE CITED

Bauer, M. L., D. L. Harmon, K. R. McLeod, and G. B. Huntington. 1995. Adaptation to small intestinal starch assimilation and glucose transport in ruminants. J. Anim. Sci. 73:1828–1838.

Calder, A. G., K. E. Garden, S. E. Anderson, and G. E. Lobley. 1999. Quantitation of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-13C amino acids as internal standards. Rapid Commun. Mass Spectrom. 13:2080–2083.

Cole, N. A., R. N. Clark, R. W. Todd, C. R. Richardson, A. Gueye, L. W. Greene, and K. McBride. 2005. Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. J. Anim. Sci. 83:722–731.

Doepel, L., G. E. Lobley, J. F. Bernier, P. Dubreuil, and H. Lapierre. 2007. Effect of glutamine supplementation on splanchnic metabolism in lactating dairy cows. J. Dairy Sci. 90:4325–4333.

El-Kadi, S. W., R. L. Baldwin VI, N. E. Sunny, S. L. Owens, and B. J. Bequette. 2006. Intestinal protein supply alters amino acid, but not glucose, metabolism by the sheep gastrointestinal tract. J. Nutr. 136:1261–1269.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.

Ferrell, C. L., R. A. Britton, and H. C. Freetly. 1992. Chronic catheterization of hepatic and portal veins in sheep. Sect. VIII A and F in Handbook of Methods of Reproductive Physiology in Domestic Animals. P. J. Dziuk and M. B. Wheeler, ed. Univ. Illinois, Urbana.

Freetly, H. C., and C. L. Ferrell. 1998. Net flux of glucose, lactate, volatile fatty acids, and nitrogen metabolites across the portal-drained viscera and liver of pregnant ewes. J. Anim. Sci. 76:3133–3145.

Freetly, H. C., and C. L. Ferrell. 2000. Net flux of nonesterified fatty acids, cholesterol, triacylglycerol, and glycerol across the portal-drained viscera and liver of pregnant ewes. J. Anim. Sci. 78:1380–1388.

Freetly, H. C., and J. Klindt. 1996. Changes in gut and liver glucose, lactate, insulin, and oxygen flux in mature ewes during mesenteric or abdominal vena cava glucose infusion. J. Nutr. 126:924–932.

Freetly, H. C., J. R. Knapp, C. C. Calvert, and R. L. Baldwin. 1993. Development of a mechanistic model of liver metabolism in the lactating cow. Agric. Syst. 41:157–195.

Gilbert, E. R., E. A. Wong, and K. E. Webb. 2008. BOARD-INVITED REVIEW: Peptide absorption and utilization: Implications for animal nutrition and health. J. Anim. Sci. 86:2135–2155.

Hanigan, M. D., L. A. Crompton, C. K. Reynolds, D. Wray-Cahen, M. A. Lomax, and J. France. 2004a. An integrative model of amino acid metabolism in the liver of the lactating dairy cow. J. Theor. Biol. 228:271–289.

Hanigan, M. D., C. K. Reynolds, D. J. Humphries, B. Lupoli, and J. D. Sutton. 2004b. A model of net amino acid absorption and utilization by the portal-drained viscera of the lactating dairy cow. J. Dairy Sci. 87:4247–4268.

Heitmann, R. N., and E. N. Bergman. 1981. Glutamate interconversions and glucogenicity in the sheep. Am. J. Physiol. 241:E465–E472.

Katz, M. L., and E. N. Bergman. 1969a. Hepatic and portal metabolism of glucose, free fatty acids, and ketone bodies. Am. J. Physiol. 216:953–960.

Katz, M. L., and E. N. Bergman. 1969b. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. Am. J. Physiol. 216:946–952.

Kreikemeier, K. K., D. L. Harmon, R. T. Brandt Jr., T. B. Avery, and D. E. Johnson. 1991. Small intestinal starch digestion in steers: Effect of various levels of abomasal glucose, corn starch and corn dextrin infusion on small intestinal disappearance and net glucose absorption. J. Anim. Sci. 69:328–338.

Lobley, G. E., D. M. Bremner, and D. S. Brown. 2001. Response in hepatic removal of amino acids by the sheep to short-term infusions of varied amounts of amino acid mixture into the mesenteric vein. Br. J. Nutr. 85:689–698.

Lobley, G. E., X. Shen, G. Le, D. M. Bremner, E. Milne, A. Graham Calder, S. E. Anderson, and N. Dennison. 2003. Oxidation of essential amino acids by the ovine gastrointestinal tract. Br. J. Nutr. 89:617–629.

MacRae, J. C., L. A. Bruce, D. S. Brown, D. A. H. Farningham, and M. Franklin. 1997. Absorption of amino acids from the intestine and their net flux across the mesenteric- and portal-drained viscera of lambs. J. Anim. Sci. 75:3307–3314.

NRC. 2007. Nutrient Requirements of Small Ruminants. Natl. Acad. Press, Washington, DC.

Reeds, P. J., D. G. Burrin, B. Stoll, and F. Jahoor. 2000. Intestinal glutamate metabolism. J. Nutr. 130:978S–982S.

Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 128:606–614.

van der Schoor, S. R., J. B. van Goudoever, B. Stoll, J. F. Henry, J. R. Rosenberger, D. G. Burrin, and P. J. Reeds. 2001. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. Gastroenterology 121:1167–1175.

Wray-Cahen, D., J. A. Metcalf, F. R. C. Backwell, B. J. Bequette, D. S. Brown, J. D. Sutton, and G. E. Lobley. 1997. Hepatic response to increased exogenous supply of plasma amino acids by infusion into the mesenteric vein of Holstein-Friesian cows in late gestation. Br. J. Nutr. 78:913–930.